Amendments to the Specification

Change(s) applied to document, /M.U./ 1/2/2013

To correct clerical errors, please amend the specification at page 57 lines ~~19 and 23~~ 18 - 27 as follows:

The coding sequence corresponding to the secreted form of the *M. tuberculosis* RP-factor (~~g1655671~~GI: 1655671; acc. no. Z81368), starting at residue D_{50}, was inserted into pET19b to generate plasmid pRPF2 (*vide infra*). Extracts of IPTG-induced *E. coli* strain HSMI74(DE3) containing pRPF2 were challenged with a poly-His antibody. A strong signal was associated with a protein which was eluted from the affinity column by 0.5M imidazole. The histidine-tagged protein from HSMI74(DE3) caused a ~~sl;ght~~slight but significant enhancement of the growth of *M. tuberculosis* H37Rv, as shown in Fig. 10. It also stimulated the growth of *M. luteus* in LMM. The control culture attained a final OD_{600nm} of 1.0, whereas cultures containing the RP-factor (1:100,000 dilution) attained a final OD_{600nm} of between 2.0 and 6.0.

Figure 10: Effect of recombinant RP-factor on growth of M. tuberculosis in Sauton medium. Sauton medium containing 0.05% Tween®-80 and 100µMol/L Na oleate + 10% (v/v) supplement (which contains, per litre, 50g bovine serum albumin, 20g glucose, 8.5g NaCl) was inoculated to an initial cell density of $31x10^3$ cfu/ml (viable count determined by plating on agar-solidified Middlebrook 7H9 medium containing 10% v/v supplement, composition as detailed above) [total count by microscopy = 10^6 cells per ml] with a 2.5 month-old culture of M. tuberculosis strain H37Ra grown in the same medium. Growth of tube cultures at 37^0C was measured by determining the OD600nm at intervals for 28 days. The undiluted concentrations of the RP-factors, Rpf (*M. luteus*) and Rpf2 (*M. tuberculosis*), employed for these experiments were ca. 10µg/ml.

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On page 45, lines 24 to 31, please amend the specification as follows:

The MPN assay was performed in a Bioscreen™ C optical growth analyzer (Labsystems, Finland) using lactate minimal medium supplemented by 0.5% lactate and 0.05% of yeast extract as a resuscitation medium. Dilutions of starved cells were made as described. 10 µl of each dilution (5-10 replicates) were added to a well containing 200 µl of either lactate minimal medium supplemented by 0.5% lactate and 0.05% of yeast extract or the same medium with fraction tested (2-20 µl). Growth (optical density) was monitored using a 600 nm filter. Plates were incubated at 30°C with intensive continuous shaking. The overall measurement period was 120h, each well being measured hourly.

On page 46, line 14 to page 47, line 13, please amend the specification as follows:

Pre-wetted DEAE cellulose was added to culture supernatant (1:10 v/v) and incubated at 4°C for lh with slow stirring. The cellulose was loaded into a column, and washed with 5 volumes of buffer 1 consisting of 10mM Tris-Cl, 1mM EDTA, 1mM DTT, 10% (v/v) glycerol, pH 7.4 with 10mM KCl. The column was eluted stepwise with 2-3 bed volumes of 0.3M KCl in buffer 1. The fraction obtained was slowly diluted with buffer 1 on ice to give a final KCl concentration of 0.08M. Forty column volumes of this fraction was then loaded onto a DEAE-~~sepharose~~ Sepharose® fast flow column (1 part of sepharose pre-equilibrated with buffer 1 containing 0.08M KC1). The column was washed with 5 bed volumes buffer 1 containing 0.08M KC1 and eluted stepwise with 3 volumes of 0.25M KC1 in buffer 1. The fraction

Figures 7A-7C: A: Purification of His-tagged RP-factor. RP-factor was expressed in E. coli HSM174(DE3) and purified as described *infra*. Shown is the SDS-PAGE profile of fractions following Ni^{2+}-chelation chromatography. The molecular weight (kDal) markers (SIGMA) were bovine serum albumin (67), ovalbumin (43), glyceraldehyde 3-phosphate dehydrogenase (36), carbonic anhydrase (30), soya bean trypsin inhibitor (20.1), and lactalbumin (14.4). Lane: 1, markers; 2, crude extract from *E. coli* containing pET19b vector; 3, crude extract from *E. coli* containing pRPF1; 4, purified recombinant RP- factor.

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At page ~~29~~ 43, please replace the paragraph spanning lines ~~36-42~~ 16 22 with the following replacement paragraph.

Figures 8A and 8B: **A:** Anti-RP-factor serum inhibits the growth of Micrococcus luteus. Bacteria were inoculated at an initial density of $5x10^5$ per ml into lactate minimal medium (LMM) and the OD_{600nm} was monitored at intervals. Growth of the cultures was monitored over 140 hours at intervals. The samples labelled LMM + Ab and LMM + control Ab contain equivalent amounts of immune and pre-immune serum, respectively. Immune serum (Ab) and pre-immune serum (control Ab) were employed at a 1:1000 dilution.

At page ~~41~~ 43, please replace the paragraph spanning lines ~~4-8~~ 29-31 with the following replacement paragraph.

Figures 9A and 9B: Part A. Blocked alignment of nine RP-factors (as explained *infra*, MtubZ94752 may be a cognate receptor). Areas of sequence identity/similarity are indicated by the shaded areas. The *S. coelicolor* gene product shown is a fragment.

suspension of starved cells (CFU 3.10^6cells.ml^{-1}, total count $1.2.10^9$cells.ml^{-1}) were added to 200 ml of LMM supplemented with 0.5 % w/v lactate and 0.05% yeast extract containing of 2 pl of each fraction in 5-10 replicates in the Bioscreen instrument. For details see Materials and Methods. **A:** absorbance at 280nm and magnitude of KCl concentration. **B:** resuscitation activity. **C:** SDS-PAGE profile of the fractions following DEAE-cellulose and Mono Q chromatography. Lanes 1, markers (94,000, 67,000, 43,000, 30,000, 20,100, 14,400); 2, fraction from DEAE-cellulose column; 3, purified preparation (fraction number 8 from the Mono Q -column). **D:** Reduction of apparent lag phase of viable cells. 10 µl of a diluted suspension of viable, stationary phase cells (viable count 20 cells) was added to 200 ml of LMM supplemented with 0.5 % w/v L-lactate and containing 2 µl of each fraction (from a different experiment to that shown in parts A and B) in 5-10 replicates in the Bioscreen instrument. The apparent lag phase was estimated by extrapolating the exponential growth line to the abscissa.

At page 28, please replace the paragraph at line 15 with the following replacement paragraph:

Figures 4A-4B: Effect of purified RP-factor on *M. luteus*.

At page ~~28~~ 41, please replace the paragraph spanning lines ~~31~~ 25 and ~~32~~ 26 with the following replacement paragraph:

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Figures 5A-5C: Detection of RP-factor-like genes in Micrococcus gluteus, *Mycobacterium smegmatis* and *Streptomyces rimosus*.

At page 28, please replace the paragraph spanning lines 6-14 with the following replacement paragraph.

Figures 6A and B : Effect of *M. luteus* RP-factor on the growth of *Mycobacterium smegmatis* (A) and *Mycobacterium bovis* (B) in batch culture as observed turbidimetrically. *M. smegmatis* was grown in broth E, to which was added RP-factor at 31 pMol/L. Cells were inoculated at a level of *circa* 200 per well, and growth was monitored in the Bioscreen instrument. *M. bovis* was grown in Sauton medium, as described in the Materials and Methods section, to which RP-factor (620 pMol/L) was either added or not. The inoculum was *circa* 1.10^5 cells.ml^{-1}, and the OD shown is the average of 10 separate determinations of 10 separate tubes.

At page 29, please replace the paragraph spanning lines 16-23 with the following replacement paragraph.

AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraph at page 1, line 3 of the application.

This application is a national stage application of ~~corresponds to~~ international patent application PCT/GB98/01619, filed June 3, 1998, and published as WO 98/55624 on December 10, 1998, which claims priority to 97113898,8 and 9811221.2, the disclosures of each which are ~~is~~ incorporated herein in ~~its~~ their entirety by reference.

Please replace the paragraph at page ~~26~~ 39, line ~~10~~ 7, with the following replacement paragraph:

Brief Description of the Drawings ~~Explanation of the Figures~~

Please replace the paragraph spanning page ~~26~~ 39, lines ~~11~~ 9, to page ~~27~~ 39, line ~~6~~ 17, with the following replacement paragraph:

Figures 1A-1E: Part A. Multiple sequence alignment of the predicted amino-acid sequences of RP-factor-like gene products from *M. luteus*, *M. tuberculosis*, *M. leprae* and *Streptomyces coelicolor*. Proteins similar to the RP-factor are derived from *M. tuberculosis* (accession nos. U38939, nt 2406-2765, and Z81368, nt 33932-34396) and *M. leprae* (accession nos. L01095, nt 12292-12759, and L04666, nt 25446-24921). The DNA sequences of interest in accession Z81 368 are also encompassed by accession AD000010. N-terminal residues corresponding to predicted Gram-positive signal sequences are underlined. The *M. leprae* L04666 sequence may also contain a short, 32 aa signal peptide.

At page ~~27~~ 40, please replace the paragraph spanning lines ~~34-37~~ 12 - 15, with the following replacement paragraph:

Figures 2A-and 2B: Part A. The sequence of the RP-factor-encoding gene and its predicted product. The nucleotide sequence is in lower case with PCR primers in bold. The predicted protein sequence is in upper case bold (single letter code). Protein and peptide microsequence data used for oligonucleotide design are in upper case italics.

Please replace the paragraph spanning page 27, line 41, to page 28, line 14, with the following replacement paragraph:

Figures 3A-3D: The elution profile of the resuscitation activity. Fractions eluted from the DEAE- sepharose column (see Materials and Methods) with 0.25 M KCl were applied to a Mono Q column which was developed with a 20ml linear gradient from 0.08 to 0.28 M KCl in 10 mM Tris-Cl buffer supplemented by 10% glycerol, pH 7.4. 10 ml of a diluted

Amendments to the Specification:

Please amend the specification as follows:

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Please delete the paragraph on page ~~17~~ 17, beginning at line ~~39~~ 4 and ending on line ~~41~~ 6, and replace it with the following amended paragraph:

The domain may comprise an alanine plus proline-rich segment, such as one or more of the amino acid motifs depicted in Figure 1D as 'A' (SEQ ID NO: 59), A, B, B' (SEQ ID NO: 63), C (SEQ ID NO: 29), 'C (SEQ ID NO:55), D, D* (SEQ ID NO: 56) and D' (SEQ ID NO: 58) (any one of which may be tandemly repeated) ~~as set out in Figure 1~~D. Motifs A, B and D are depicted in Figure 1D with brackets around two amino acids, to indicate that the motifs define sequences that include a choice of one or the other of the two amino acids within the brackets as follows:

A = appvela[av]ndl (SEQ ID NO: 62);

B = paplgeplpaapa[de]l (SEQ ID NO: 60); and

D = appapa[de][lv] (SEQ ID NO: 61).

Please delete the current Sequence Listing and insert in its place the substitute Sequence Listing submitted herewith.